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ABSTRACTS OF PAPERS AND DISCUSSION

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Immunologic Mechanisms in Experimental Vasculitis

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This presentation deals with several experimental models in which immunologic reactions are associated with vasculitis. The models chosen have readily identifiable antigen-antibody reactions and may be analogous to some human diseases.

In serum sickness, the essential immunologic event is the interaction between circulating antigen and newly formed antibody, initially in an environment of antigen excess, and finally in one of antibody excess. Coincident with the antigen-antibody reaction are inflammatory and necrotizing changes in small and medium-sized arteries, particularly of the heart, and an endothelial proliferation in the glomerular capillaries. Fluorescent antibody observations reveal the presence of antigen and probably antibody in these vascular lesions.

The essential immunologic feature of the Arthus reaction is the interaction of antigen with antibody in the walls of small vessels. One of the reactants must be in the circulation and the other injected locally to insure the immunologic reaction within the vessel wall. Fluorescent antibody studies reveal a sub-endothelial antigen-antibody deposit in small vessels in the Arthus site. In the absence of circulating polymorphonuclear leukocytes, this antigen-antibody deposit appears to elicit little or no inflammatory reaction. In the presence of polymorphonuclear leukocytes, the polys infiltrate the affected vessels and phagocytize the antigen-antibody complexes, which they then catabolize and/or carry off. Thus, this phlogogenic stimulus, initiated by antibody-antigen interaction, is fully expressed only in the presence of polymorphonuclear leukocytes.

A chronic glomerulonephritis, morphologically and clinically quite similar to its human counterpart, can be produced in rabbits by daily intravenous injections of foreign serum proteins. This course of antigen injections results in a prolonged antigen-antibody interaction in the circulation. Glomerulonephritis develops in those rabbits which produce some but not enough antibody to combine completely with and cause the elimination of all circulating antigen. These relatively poor antibody producers have a continual antigen-antibody reaction occurring in the circulation in an antigen-excess environment. Fluorescent antibody studies indicate the heavy deposition of antigen and probably antibody along the basement membranes of affected glomeruli. Electron microscopic observations reveal these antigen-antibody deposits to be in contact with the outer surface of the basement membrane, between the membrane and the epithelial cell cytoplasm. Similar electron-dense deposits have been observed in human chronic glomerulonephritis.

Studies on Transplantation Immunity

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The role of circulating antibody in the rejection of homografts is still not generally recognized, in spite of the growing number of reports of the existence of cytotoxic antibodies in the serum of grafted animals and of the passive transfer of anti-graft immunity by such serum. Much of the argument against the participation of serum antibodies is based on early work with cell-impenetrable chambers which indicated that host cells, but not antibodies, are involved in graft destruction. More recent work has indicated that grafts in such chambers can indeed be destroyed by antibody in the absence of host cells. While immunity to grafts of dissociated normal or malignant cell suspensions has been readily transferred with serum, such has not usually been the case with orthotopic homografts (of skin, for example). Preliminary evidence has been obtained that in such vascularized grafts there exists a blood-graft barrier to the passage of antibodies, and that graft destruction may be accomplished by cytotoxic antibodies only after some increased permeability of blood vessels is produced, perhaps by a local delayed hypersensitivity reaction.

DISCUSSION

JOHN G. KIDD: The concepts and phenomena of immunology have in the past given rise to much food for thought and to many problems of interest for pathologists. We have heard this evening from two investigators who are now working effectively in this field. Their papers show clearly, it seems to me, that newer techniques of immunology, when industriously applied to problems having to do with alterations in tissues and cells *in vivo,* can still disclose findings having at once wide biological implications and special interest and importance for those who are interested in the causation and nature of disease.

Dr. Dixon and his group are to be congratulated upon their mastery of the fluorescent antibody technique—first developed by Coons and Kaplan—and for their critical application of this technique and other immunological procedures to the study of experimental vasculitis and glomerulonephritis. With respect to the nature of the induced arteritis, it seems to me that they have in this lesion a very close experimental model of the acute stages of naturally occurring periarteritis nodosa—though this disease has intruded itself somewhat less often upon our thoughts during the past several years than it used to do some years ago when the American public was consuming several hundred tons of sulfonamides per annum. Furthermore, the reversible glomerulonephritis associated with induced serum sickness, shown in Dr. Dixon's first group of slides, although clearly unlike the lesion of chronic progressive glomerulonephritis that we see at the postmortem table, may still provide something of a counterpart for the lesion of naturally occurring acute glomerulonephritis, for it is well known that most patients who develop this disease recover from it in due course.

By means of the fluorescent antibody and

other immunologic techniques in combination with light and electron microscopy, Dr. Dixon has shown quite precisely the localization of antigen and antibody—presumably joined together in the form of complexes—at the sites of injury in these induced acute lesions. His findings bear upon the causation and pathogenesis of several experimental diseases and their naturally occurring phototypes.

In his more recent studies, as illustrated in his second group of slides, Dr. Dixon has produced a progressive chronic glomerulonephritis which, to my eye at least, has all the earmarks of chronic active (Type I) glomerulonephritis as we see it in human beings. I question whether he would have achieved this noteworthy result had he not been at once an experienced pathologist with facilities for work on an extensive scale, and at the same time a competent immunologist with due regard for the values of quantitation. For, as you will recall, when he gave bovine albumin repeatedly over long periods of time to rabbits that were good antibody producers, and thus stimulated with relatively little antigen the formation of very large quantities of antibody, the lesion did not develop; and the same was true when he gave the antigen in excessive amounts to animals that were unresponsive or poor producers of antibody. The chronic nephritis developed only in those rabbits in which complexes composed of more or less equivalent amounts of antigen and antibody were presumably present in the circulating blood during considerable periods of time. I am sure we shall all await with interest the publication of these findings in detail, and their inevitable extension in numerous directions.

Now Dr. Dixon has not travelled all the way from Pittsburgh merely to hear nice things said of his work. He expects, I am sure, at least one captious remark, and I am prepared to deliver this. But first I wish to commend him further for not using the term "collagen disease" in his presentation this evening. It seems to me high time that pathologists generally—at least those who have regard for precision in terminology and nomenclature[1]—should again follow Dr. Klemperer's lead[2] and re-examine the basis upon which they have employed this term during the past several years. Recent observations on the nature of disseminated lupus, including those of Dr. Klemperer, lead us away from the notion that collagen is primarily injured in this disease[3]. Furthermore, the observations of George Murphy indicate that heart muscle, not collagen, is primarily injured in rheumatic disease[4], and the observations of other workers provide more than an indication that what has so long and glibly been called fibrinoid is, in large part at least, merely fibrin[5, 6]. In this latter relation I recall the extraordinary reserve that Dr. Klemperer has always displayed toward the terms "fibrinoid degeneration" and "fibrinoid necrosis"; he has been at once vigorous, articulate, and logical in pointing out that these terms lack specificity and precise meaning[3]. And this leads me to my captious comment: Twice this evening, within earshot of us all, Dr. Dixon has pointed to "fibrinoid" in his acute lesions. I hope that others will not conclude from Dr. Dixon's reference to fibrinoid that collagen is primarily injured in the lesions he has produced; also that Dr. Dixon can manage in due course to define precisely the point of injury.

Dr. Stetson has concerned himself mainly with the mechanisms whereby mammalian organisms react against transplanted tissue cells and bring about their death. As he has pointed out, this is a problem that has interested many investigators during the past 50 years. It is also a problem having quite wide implications—bearing, for example, upon the nature of genes and of antigens, upon the phenomena of hypersensitivity, and, more remotely perhaps, on the manifestations of auto-immune states. Furthermore, during the past 20 years there has been a renewal of interest in this problem; and from recent work in this field a new and fundamental phenomenon—namely, *immunological tolerance*—has come as a by-product.

My own interest in this problem has centered around immunity to transplanted cancer cells. Much of the early work in this field was done with these as materials. Some 50 years ago, when cancer began to be studied intensively in laboratories all

over the world, only random-bred mammals were available, and investigators who then undertook to transplant a spontaneous tumor in this species found it necessary to use dozens of hosts in order to get a take in one or a few animals, and they were able to keep the tumor going only by using large numbers of hosts during several serial transfers. Even after this critical period, many of the grafts grew only briefly and then regressed, the implanted hosts being immune thereafter. Now it has become known to everyone that genetically determined antigens, present in cells of the graft but absent from tissues of the host, stimulate in the host a specific immune reaction which somehow overcomes the graft. Yet the nature of the immune reaction and the mechanism whereby the grafts are overcome have remained uncertain to the present day; much controversy still centers around the question whether humoral antibodies or "immune" lymphocytes effect the result, as Dr. Stetson has told us.

Such knowledge as we now have of immunity against transplanted tissues has not by any means burst suddenly upon us. Many years ago, Dr. Rous showed that immunity against transplanted cancer cells can be induced artificially by injecting either living cancer cells or living embryonic (non-neoplastic) cells into alien hosts, the immunity thus induced being effective against grafted normal tissues as well as against grafted neoplastic tissues[7]. Dr. William Woglom, who used to come often to the meetings of this Society, wrote two detailed reviews on immunity to transplanted cancer cells, one published in 1913 and the other in 1929. In the latter publication, which deals with observations recorded in more than 600 papers, Woglom noted particularly that all attempts theretofore to demonstrate humoral antibodies as responsible for immunity to transplanted cancer cells had failed, and he concluded that a "generalized host resistance" of unique sort was probably responsible when tumor cells were overcome in alien hosts[8]. Some years later, upon reviewing the work of Lumsden and the earlier work of Gorer on isoantibodies in relation to immunity to transplanted cancer cells, I concluded that the isoantibodies that they had found in the blood of resistant hosts might well prove to be epiphenomena in relationship to the regression of transplanted tumors[9]. More recent work makes it plain that the effects of isoantibodies in this relationship remain still uncertain[10, 11]. By contrast, Da Fano[12] and J. B. Murphy[13] long ago convinced themselves that lymphocytes are probably responsible for the regression of grafted tumors in resistant and immune hosts.

And this brings me to the contentious aspect of what I have to say about Dr. Stetson's presentation. Some years ago, after inbred animals had become readily available, Dr. Toolan and I, with Dr. Ellis participating in a part of the work, made a histologic study of regressing C3H mammary carcinomas in Strain A mice—this with the aim of learning, if we could, the means whereby cancer cells are overcome in alien hosts[14]. We found that cells from a C3H mammary carcinoma, implanted into the subcutaneous tissues of animals of that inbred strain, began to proliferate within 24 to 36 hours following implantation and continued to do so thereafter until death of the host, without calling forth any inflammatory response or being influenced in any discernible way by immunological factors. When we put these cells into albino (Strain A) mice, we found that they also grew during the first five days following implantation, and precisely as in C3H mice. Quite regularly on the fifth or sixth day, however, lymphocytes began to appear about the growths in the alien Strain A mice. The lymphocytes soon became numerous, and they promptly began to infiltrate the small nodules of proliferating tumor cells from the periphery inwards. As the lymphocytes established intimate contact with the proliferating tumor cells, the latter began to die one by one, the process proceeding quite rapidly until the last tumor cell had died, usually before the 12th day. Furthermore, as they died, the tumor cells exhibited an interesting sequence of cytologic changes which was wholly unlike that to be seen in tumor cells dying from other causes—e.g., as the result of anoxia, or when heated. When a Strain A mouse had overcome its first C3H tumor and was later reimplanted, the lymphocytes accumulated

much more promptly. Within 24 hours they began to collect in considerable numbers about the implanted lymphoma cells, and within 48 to 72 hours the lymphocytes were not only exceedingly numerous but also closely approximated to the proliferating tumor cells. Soon the latter began to exhibit the distinctive cytologic changes, and within five or six days following implantation they were all dead[14]. In further experiments we procured blood serum and suspensions of lymph node cells from immune Strain A mice, often from animals that had been repeatedly reimplanted. The serum had no effect on the tumor cells *in vitro*, whereas suspensions of cells from the lymph nodes always acted powerfully against them. Hence my own experience has led me to the conclusion[14]—previously reached by Da Fano[12] and by Murphy[13] and subsequently arrived at by Mitchison[15] and by Weaver, Algire and Prehn[16]—that "immune" or "sensitized" lymphocytes probably overcome tumor cells when these are transplanted to resistant hosts[13]. It was inevitable that we should see a parallel between these observations and those of Landsteiner and Chase, which indicate that cells are responsible for certain of the manifestations of hypersensitivity[17, 18].

In spite of my own experience in this relation, it seems to me wholly appropriate that Dr. Stetson should question the conclusion that lymphocytes bring about regression of tissues transplanted in alien hosts, and that he should mobilize from his own experiments and from the observations of others such evidence as he can find to support the inference that humoral antibodies are responsible. For after all, to paraphrase a saying of Josh Billings: a difference of opinion can lead to experimentation, and perhaps, indeed—to discovery. Surely, in a field as complicated as this one is, the repetition of work and the application of differing points of view are much to be desired if the points at issue are to be resolved.

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